Allelopathy and Autotoxicity in Three Eastern North American Ferns

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Undoubtedly gametophytes are the most vulnerable stage in the fern life cycle. Although individual sporophytes produce from 50 to 100 million haploid spores (Shaver, 1954), few of the small, delicate gametophytes, which in most ferns are less than 1.25 cm wide, survive and produce sporophytes. Conditions governing spore germination are critical to gametophyte establishment. Conway (1953) found that, in Scotland, few gametophytes or young sporophytes of *Pteridium aquilinum* occurred under field conditions, despite heavy spore production by mature plants. This suggests that the gametophyte and young sporophyte stages may be limiting in the establishment of this species. Many studies (cited in Hill, 1971) suggest that fern gametophytes and sporophytes can possess quite different habitat requirements and that one stage of the life cycle can be more sensitive than the other to physical and biological conditions, and so limit the success of the species.

Although environmental (abiotic) conditions are extremely important, biotic conditions, particularly those created by the sporophytes or gametophytes, may also be of critical importance in the establishment of new individuals of the same or another species. Therefore, these factors may regulate both population density and community composition. Chemical inhibition of one plant by another, or allelopathy, has been known for over a century (Muller, 1966) and has been much studied in flowering plants and conifers. The phenomenon of antibiosis is also well known to microbiologists. However, relatively little research has been devoted to plant inhibitors produced by non-seed plants, other than microorganisms (Rice, 1967).

Bohm and Tryon (1967) reported that many species of ferns produce phenolic compounds. They examined 46 species for the presence of hydroxylated cinnamic and benzoic acids and found a basic complement of cinnamic acids (p-coumaric, caffeic, and ferulic) in the ferns they tested. Also generally present were p-hydroxybenzoic, protocatechuic, and vanillic acids. Sinapic, syringic, and o-coumaric acids were reported to be less common. In a follow-up study, Glass and Bohm (1969) found similar phenolic compounds in 46 additional species. The presence of a basic complement suggests that the well established pathways of phenolic metabolism in the seed plants also function in ferns.

Many of the phenolic compounds found in ferns are known to be allelopathic in many species of higher plants, either directly or indirectly, such as after microbial decomposition (Rice, 1974). Most phenolic acids are at least slightly soluble in water. With the increasingly acidic rainfall in the northeastern United States (Likens et al., 1970; Bormann & Likens, 1977), weak organic acids such as the phenolics may be leached quite readily either from the leaves during the growing season or from senescent plants. A wide variety of organic and inorganic sub-

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stances known to be allelopathic are capable of being leached from the leaves of a number of species of higher plants (Tukey, 1966, 1969; Rice, 1974).

Of the few allelopathic studies concerned with ferns, most have involved *Pteridium aquilinum*. Del Moral and Cates (1971) reported that aqueous leaf extracts and litter extracts of *P. aquilinum* inhibit seeds of *Hordeum vulgare*, *Bromus tectorum*, and *Pseudotsuga menziesii*. Gliessman and Muller (1972) found that *P. aquilinum* inhibited germination and subsequent radicle growth of seeds of *Bromus rigida* and *Avena fatua*. The toxic principle was detected in the fern leaf leachates. Because the toxic principle was transported by some form of precipitation, water-soluble phenolic acids were suspect, and cinnamic acid was tentatively identified. In another study, Stewart (1975) found that water-soluble extracts from Western Bracken (*P. aquilinum* var. *pubescens*) delayed germination of *Rubus spectabilis* seeds and inhibited germination of *R. parviflorus* seeds, but had no effect on the seeds of *Pseudotsuga menziesii*.

Glass (1976) prepared a solution of phenolic acids having the same composition as was detected in the soil associated with the roots of *P. aquilinum* and tested its effect on the growth of barley, wheat, oats, rye, rye-grass, barley grass (*Hordeum murinum*), clover, and *Agropyron repens*. Growth was inhibited in all species investigated except *A. repens*. Whether *P. aquilinum* sporophytes in some way inhibit the germination of their own spores or those of another species of fern was not investigated in Glass's or any other study reported in the literature.

Davidonis and Ruddat (1973) found that *Thelypteris normalis* sporophytes inhibit the growth of *T. normalis* gametophytes, as well as those of *Pteris* and *Phlebodium*. The inhibitors, which they termed thelypterins a and b, were similar in many ways to indoleacetic acid (IAA) and were found to be exuded from sporophyte roots. Davidonis and Ruddat (1974) also reported that gametophytes grown in the immediate vicinity of a mature sporophyte of *T. normalis* had a reduced number of cells and an altered gross morphology. They observed the greatest inhibition of *T. normalis* gametophytes when the thelypterins were added before spore germination had occurred.

Davidonis (1976) reported that *T. noveboracensis*, *Pteris multifida*, and *P. vittata* also contained thelypterins. Since the thelypterins were detected in leaf diffusates of *T. normalis* and *T. noveboracensis*, she postulated that foliar leaching may be one mechanism by which these inhibitors are released into the environment.

Petersen (1976) found that *Dryopteris intermedia* and *Osmunda cinnamomea* gametophytes inhibited each other's development when grown together in culture. He also reported that gametophytes of these two species "lock" each other into perpetual juvenility. Petersen was unable to isolate and indentify the inhibitory substances since they occurred in very small amounts or were highly volatile.

Horsley (1977) reported that the presence of *Dennstaedtia punctilobula* and *T. noveboracensis* sporophytes were correlated with reduced numbers of *Prunus serotina*, *Acer rubrum*, and *A. saccharum* seedlings under field conditions.

Very limited research has been conducted either to detect possible allelopathic interactions between species of ferns or to demonstrate the inhibition of one

generation by the other within a species (autotoxicity). It is not known whether allelopathy and autotoxicity are widespread in natural fern communities. Davidonis and Ruddat (1973, 1974) and Davidonis (1976) examined a small number of species for inhibitors, but in many instances the species tested for allelopathic interactions were those that are maintained in greenhouses, and not those which occur together in natural situations. Petersen (1976) has shown that fern gametophytes of one species produce substances that inhibit gametophytes of others, the classic allelopathic inhibition of one species by another. Under field conditions, however, the dominant and normally perennial sporophytes probably produce much greater quantities of allelopathic compounds capable of affecting spore germination or gametophyte growth than would the much smaller gametophytes. Petersen's research suggests that sporophytes might also inhibit gametophytes, as they were found to have produced the same flavonoids, but no experimental evidence to support such a hypothesis was presented.

There are reports suggesting allelopathy between fern generations in both natural populations and the greenhouse. In greenhouses, potted ferns often produce large numbers of spores, but gametophytes are seldom found growing on the soil surface directly underneath the sporophytes. It is also unusual to find gametophytes growing underneath or even near mature sporophytes under field conditions, even when spore production is heavy.

In this research, aqueous leaf extracts, leaf leachates, and leaf litter infusions from *Osmunda cinnamomea, O. claytoniana*, and *Dennstaedtia punctilobula* were examined for their allelopathic and autotoxic potential. Leaves of all three species were also tested for the production of volatile inhibitors.

All three species occupy much the same habitat, although *O. cinnamomea* is often found in moister areas than the other two. The geographical ranges of all three species also are approximately the same, and all are native and common in the northeastern United States. Although these three species occupy almost identical habitats, they seldom occur in close proximity to one another within a given area.

MATERIALS AND METHODS

Mature spores of *O. cinnamomea*, *O. claytoniana*, and *D. punctilobula* were collected in 1976 and 1977 from wild populations which were located in Sandystone Township, Sussex County, New Jersey, and in the Town of Goshen, Addison County, Vermont.

Spore-bearing leaves of both species of *Osmunda* were collected during the first two weeks of May from New Jersey populations and during the last week of May and first week of June from Vermont populations. Sporangia were allowed to dehisce at room temperature. Spores were refrigerated at 4.4° C as soon as possible after they were shed from the sporangia. Although *Osmunda* spores contain chlorophyll and remain viable for only a few days at room temperature (Cobb, 1963), they will retain their viability for over a year if refrigerated (Stokey, 1951).

Spore-bearing leaves of *D. punctilobula* were collected during the last two weeks of July from the New Jersey populations and during the first week of

August from Vermont populations. After dehiscence of the sporangia, spores were stored dry and at room temperature. The spores of *D. punctilobula* lack chlorophyll and have a thicker, more resistant spore wall than *Osmunda* spores, and so require no refrigeration to remain viable.

Fresh fronds from all three species were used to prepare aqueous extracts and leachates. They were collected from both Vermont and New Jersey populations throughout the growing season. Fronds were refrigerated immediately after collection and were then stored at 4.4° C. The extracts and leachates were prepared from the fronds within three or four days after collection.

All aqueous leaf extracts were prepared by first cutting into pieces 5 g of leaves (fresh weight) and then placing the pieces in 80 ml of distilled water. The leaves were boiled briefly (3 min) to stop enzyme activity and, it was hoped, to destroy some of the microorganisms which might later contaminate cultures. After boiling, the leaves were ground in a Virtis blender for 5 min. The ground mixture was then vacuum filtered and the resulting aqueous extract was brought to volume with distilled water (100 ml distilled water for each 5 g of ground leaves). Extracts were stored at 4.4° C until they were used to prepare cultures, usually within 3–5 days.

Leaf leachates for each species were prepared by placing fronds (2 thick) on 1.5 mm mesh screen which was placed on top of a rectangular plastic box. Fronds having an area of ca. 630 cm^2 were then misted with 300 ml of distilled water. The droplets of water falling from the leaves were collected in the large plastic box. Leachates were stored at 4.4° C until used.

Litter infusions were prepared by placing 10 g of chopped dry frond litter in a 500 ml beaker. Weekly additions of 100 ml of distilled water were made for a period of 3 weeks. Beakers were placed in direct light in the greenhouse. Filter paper was placed on top of each beaker to prevent external contamination. After 3 weeks, the aqueous portion of the infusion was decanted and vacuum filtered. The filtered liquid was used to prepare the experimental cultures.

The methods used to prepare cultures were based on those reported by Munther (1975). All cultures using extracts, leachates, and infusions were prepared as follows: plastic seed germinators (clear plastic boxes measuring 11 × 11 × 3 cm) were filled with 0.138 l (dry) of autoclaved horticultural grade (medium) vermiculite, and various treatment solutions were added to the vermiculite. The treatment solutions contained two parts of extract, leachate, or infusion and one part of 2X Hoagland's no. 1 solution plus trace elements (Hoshizaki, 1975). Fern spores generally require only moisture to germinate (Miller, 1968), but a nutrient solution was added because the cultures were examined over an extended time, and nutrients are necessary after the first few cell divisions of germinating spores. A total of 75 ml of solution was added to each experimental box. In controls, distilled water was mixed with Hoagland's solution instead of the extracts, leachates, or infusions. Fern spores were sown on the upper surface of pieces of unglazed clay pots placed on top of the vermiculite, 3 per germinator. New pots were used, and all were from the same manufacturer's lot. The pots, broken into pieces of approximately equal size, were boiled vigorously in water for 15 min three separate times, using fresh water for each boiling to remove the impurities.

Cultures were placed under 285–315 ft-c of illumination provided by alternately placed 40-watt plant grow (G.E.) and cool white fluorescent tubes with a photoperiod of 14 hrs. The light intensity used was based on field observations obtained with a light meter and on a recommendation reported by Miller and Miller (1961). Temperature was maintained at 22–24° C.

Each culture was checked daily after sowing until the first spores began to germinate. At that time, a count was made to determine percent germination and then repeated every other day for thirteen days. Since experimental data indicated that the last two counts were not appreciably different, the time was later reduced to 11 days. The criterion used to determine when spore germination had occurred was the appearance of the rhizoid following the first division of the spore, not just the uptake of water as shown by swelling. All counts were made under low power (100×) of a light microscope, and a mechanical counter was used to record the number of germinated and non-germinated spores within a randomly selected microscopic field. One count was taken from each chip in the box. All experiments were conducted in triplicate, using three germinator boxes for each replicate.

To test for the production of volatile compounds by the fronds of each of the three species, 30 g of fresh fronds were placed in a 16 × 31 cm plastic box. Also within this plastic box were 9-cm petri dish bottoms containing culture medium similar to that used in the other experiments. Each plate contained 0.091 l (dry) of vermiculite plus 50 ml of control solution on top of which were the pieces of clay pot with spores. The box was sealed with clear plastic tape. For controls, 30 g of cotton moistened with water was used in place of the fresh fronds. This method was similar in principle to that described by Muller (1966), and allowed any volatile compounds produced by the fronds to concentrate in the closed atmosphere of the box. The only contact between the fronds and the spores was through the air. The sealed boxes were placed under 300 ft-c of light for 14 days, after which counts were taken to calculate percent germination. All experiments were conducted in tripicate.

The replicated means of percent germination resulting from treatment with leaf leachates and extracts were compared statistically using Duncan's multiple range test (Duncan, 1955; Steele & Torrie, 1960), with replicated control means, for every possible autotoxic and allelopathic interaction (Munther, 1978). Separate comparisons to controls were made using the litter infusion treatment means. Only the means from the first (day 1) and last (day 11 or 13) counts were used for statistical analysis. These statistical comparisons were therefore based on a minimum (the presence of any germinated spores in any replicate of a species and treatment) and a maximum (all spores capable of germination from a variable population) level of germination for every possible interaction. Replicated volatile treatment means were compared to the controls in a similar manner; however, only one series of means representing the maximum level of germination was used in these comparisons, since it was not possible to obtain a minimum level due to the design of the experiments.

RESULTS AND DISCUSSION

In the following discussion, compounds produced in the leaves of a species which inhibit the germination of spores of the same species will be termed "autotoxic." The inhibitory effects on spores of another species will be termed "allelopathic."

Spores of both *Osmunda* species began to germinate in five or six days in Vermont and New Jersey populations. *Dennstaedtia* spores generally took longer to begin germination. Those from Vermont required at least 11 days, and those from New Jersey required at least 8 days.

TABLE 1. EFFECTS OF AQUEOUS LEAF EXTRACTS AND LEACHATES ON SPORE GERMINATION AND EARLY GAMETOPHYTE GROWTH IN *DENNSTAEDTIA* AND *OSMUNDA*.

Species		*Vermont*				*New Jersey*			
Spores	*Leaves*	*Leachate*		*Extract*		*Leachate*		*Extract*	
		FC	LC	FC	LC	FC	LC	FC	LC
		Autotoxic Interactions							
O. cinn.	O. cinn.	+	+	+	+	−	−	−	−
O. clay.	O. clay.	−	−	−	+	−	−	−	−
D. punc.	D. punc.	−	−	+	+	−	−	+	+
		Allelopathic Interactions							
O. cinn.	O. clay.	−	−	+	+	−	−	−	+
O. cinn.	D. punc.	−	−	+	+	+	+	+	+
O. clay.	O. cinn.	−	−	−	−	−	−	−	−
O. clay.	D. punc.	−	−	−	−	−	−	−	−
D. punc.	O. cinn.	−	−	+	+	−	−	−	−
D. punc.	O. clay.	−	−	+	+	−	−	−	−

FC = first count; LC = last count
+ = statistically significant inhibition (0.05 level); − = no significant inhibition

In the Vermont populations, all species exhibited some degree of autotoxicity (*Table 1*). In each species, the leaf extract was found to inhibit germination significantly, particularly in the last count. In *O. cinnamomea,* leaf leachates also significantly inhibited spore germination, and the inhibition was nearly as great as that caused by the extracts (*Table 3*). This differed considerably from the results obtained from the New Jersey populations, where only one species, *D. punctilobula*, was found to be significantly autotoxic (*Table 1*). In this case, the inhibition was caused by the extract.

Significant allelopathic interactions also were found in the Vermont populations. Leaf extracts of both *O. claytoniana* and *D. punctilobula* inhibited spores of *O. cinnamomea*, significantly lowering percent germination (*Table 1*). Inhibition of germination was quite severe, particularly as revealed in the final counts (*Tables 4* and *5*). Spores of *D. punctilobula* were inhibited by leaf extracts of *O. cinnamomea* and *O. claytoniana* (*Table 1*). Inhibition in this case also was quite marked, especially by extracts of *O. claytoniana* (*Table 4*). Aqueous leaf extracts of *O. claytoniana* often severely inhibited spores of the other two species, but extracts of both *O. cinnamomea* and *D. punctilobula* had little effect on *O. claytoniana* spore germination (*Table 1*). Leaf leachates of the three species tested produced no significant allelopathic inhibition of spore germination.

The number of significant allelopathic interactions was lower in the New Jersey populations than in the Vermont populations (*Table 1*). Spores of *O. cinnamomea* were inhibited by leaf leachates of *D. punctilobula*, which was the only significant allelopathic inhibition by leaf leachates in either the New Jersey or Vermont populations (*Tables 1* and *5*). The inhibition produced by the leachates was not so severe as that produced by the extracts (*Table 5*). Also in New Jersey, germination of *O. cinnamomea* spores was inhibited by leaf extracts of both *O. claytoniana* and *D. punctilobula* (*Table 1*). *Osmunda cinnamomea* and *O. claytoniana* leaf extracts had little effect on germination of *D. punctilobula* spores (*Table 1*). This result is quite different from that obtained for the Vermont populations (*Table 1*). However, as in the Vermont populations, spores of *O. claytoniana* were unaffected by leaf extracts (or leachates) of both *O. cinnamomea* and *D. punctilobula* (*Table 1*). Nevertheless, in the majority of cases, the Vermont and New Jersey populations reacted quite differently in terms of the number and type of allelopathic and autotoxic interactions.

TABLE 2. EFFECT OF LEAF LITTER INFUSIONS ON SPORE GERMINATION AND EARLY GAMETOPHYTE GROWTH IN NEW JERSEY POPULATIONS OF *DENNSTAEDTIA* AND *OSMUNDA*.

Species		*Effect of litter infusion*	
Spores	*Leaves*	*FC*	*LC*
	Autotoxic Interactions		
O. cinn.	O. cinn.	+	−
O. clay.	O. clay.	−	−
D. punc.	D. punc.	−	−
	Allelopathic Interactions		
O. cinn.	O. clay.	−	−
O. cinn.	D. punc.	−	−
O. clay.	O. cinn.	−	−
O. clay.	D. punc.	−	−
D. punc.	O. cinn.	−	+
D. punc.	O. clay.	−	−

FC = first count; LC = last count
+ = statistically significant inhibition (0.05 level); − = no significant effect

Leaf litter infusions of New Jersey material did not inhibit germination greatly (*Table 2*). *Dennstaedtia punctilobula* spores were inhibited by *O. cinnamomea* leaf litter infusions, the only allelopathic inhibition caused by treatment with litter infusions. The only species exhibiting autotoxicity was *O. cinnamomea* (*Table 2*), in which the litter infusion merely delayed germination, since there was no inhibition in the last count.

Two types of inhibition were observed in spores treated with extracts and leachates. Spores of all three species imbibed water readily when treated with leachates or extracts, but spores treated with extracts often did not divide. This indicates that the inhibitor was able to enter the spore through the wall and to prevent the first division. This type of inhibition causes a low percentage of spores to germinate, is evident in the first count when compared to the controls, and

continues through the last count (*Table 1*). This was the most common type of inhibition. In the second type, several divisions occurred and percent germination was not affected in the first count (see *O. claytoniana*, autotoxicity, *Table 1*). The inhibitor in this instance did not affect germination, but acted on the several-celled stage (young gametophyte). In the later counts, if a spore had germinated but had died (chlorophyll lost) at the several-celled stage, it was counted as not germinated. Therefore, the last count measures the inhibitor's effect on early gametophyte growth and development. The presence of this type of inhibition can be recognized only at the last count.

When leaf extracts caused significant inhibition of germination, gametophyte development from spores which germinated usually was affected, with the gametophyte usually arrested in the filamentous stage and little or no further growth occurring. Leaf leachates usually did not cause this type of response. Gametophytes resulting from spores which did germinate underwent normal development, but often exhibited somewhat slower growth than did the controls.

TABLE 3. AUTOTOXIC EFFECTS OF LEAF LEACHATES AND EXTRACTS EXPRESSED AS A PERCENTAGE OF SPORE GERMINATION AND EARLY GAMETOPHYTE GROWTH IN VERMONT POPULATIONS OF *O. CINNAMOMEA*. STATISTICAL ANALYSIS USING DUNCAN'S MULTIPLE RANGE TEST WITH A 0.05 SIGNIFICANCE LEVEL. (ALL EXTRACT AND LEACHATE MEANS SIGNIFICANTLY DIFFERENT FROM ALL CONTROL MEANS.)

First count ($s_{\bar{x}}$ = 5.421)

Means	E1	L1	E3	L3	E2	L2	C1	C3	C2
s=	4.7	2.4	4.3	4.8	4.0	10.3	19.9	14.2	2.7
$\bar{x}$=	14.7	15.6	16.8	17.2	17.8	18.1	52.1	58.2	65.5

Last count ($s_{\bar{x}}$ = 5.034)

Means	E3	E1	E2	L2	L3	L1	C1	C3	C2
s=	7.1	4.4	5.3	14.4	11.4	5.5	8.1	10.2	7.0
$\bar{x}$=	9.1	10.1	14.0	23.6	26.3	48.8	76.8	80.5	81.3

$s_{\bar{x}}$ = standard error (of the mean); s = standard deviation; $\bar{x}$ = mean percent germination
C1–3 = control means; L1–3 = leachate treatment means; E1–3 = extract treatment means

Phenolic compounds are known to inhibit ion uptake in flowering plants through reversible alterations in membrane permeability (Glass, 1973, 1974) and may affect IAA metabolism. The spore wall may prevent the phenolic inhibitor from entering the spore, with the first divisions of the spore occurring on stored reserves. By the time the gametophyte reaches the several-celled stage, the phenolic inhibitor may prevent ion uptake sufficiently to inhibit growth or further cell division.

The effects of the extracts on spore germination and on early gametophyte stages were much greater than the effects of the leachates in almost every experiment. While experimental effects of the leachates may be easily extrapolated to field conditions, the extracts present an enigma. While nothing directly paralleling an extraction procedure exists in nature, experimental results obtained using ex-

tracts do possess some validity in determining the phytotoxic potential of plants under laboratory conditions. Extracts may concentrate compounds which are leachable and concentrated in the soil under field conditions. However, some compounds present in an extract may not be leached from healthy, growing tissue, and many substances can be altered by the extraction procedure itself. On the other hand, substances that are not normally leached from such tissue can be released by senescent tissue quite readily (Gliessman & Muller, 1972; Stewart, 1975). Various forms of stress, such as temperature extremes, drought, attack by pathogens, or mechanical injury also increase the leachability of metabolites from foliage (Tukey, 1966, 1969; Rice, 1974). Soils also vary in their composition, structure, moisture content, pH, and the kinds of microorganisms present. Thus, they would have different affinities for various inhibitors, or may even render them inactive or increase their activity due to microbial decomposition (del Moral & Cates, 1971; Rice, 1967, 1969; Wang et al., 1967).

TABLE 4. ALLELOPATHIC EFFECTS OF *O. CLAYTONIANA* LEAF EXTRACTS AND LEACHATES EXPRESSED AS A PERCENTAGE OF SPORE GERMINATION AND EARLY GAMETOPHYTE GROWTH IN VERMONT POPULATIONS OF *O. CINNAMOMEA* AND *D. PUNCTILOBULA*. STATISTICAL ANALYSIS USING DUNCAN'S MULTIPLE RANGE TEST WITH A 0.05 SIGNIFICANCE LEVEL. (ALL EXTRACT (NOT LEACHATE) MEANS SIGNIFICANTLY DIFFERENT FROM ALL CONTROL MEANS.)

O. cinnamomea

Last count ($s_{\bar{x}}$ = 8.450)

Means	E2	E3	E1	L2	L1	L3	C1	C3	C2
s=	14.1	18.0	27.2	19.0	8.7	3.5	8.1	10.2	7.0
$\bar{x}$=	12.2	22.1	39.4	43.6	54.8	58.4	76.8	80.5	81.3

D. punctilobula

First count ($s_{\bar{x}}$ = 4.618)

Means	E1	E3	E2	L1	L2	C1	C3	L3	C2
s=	3.8	5.8	3.3	13.7	3.8	9.1	14.5	4.5	1.3
$\bar{x}$=	15.1	18.5	28.0	36.0	41.9	47.5	49.7	52.7	54.5

Last count ($s_{\bar{x}}$ = 4.965)

Means	E1	E3	E2	L1	L2	L3	C3	C1	C2
s=	5.1	12.5	15.6	2.3	10.9	5.3	6.4	2.9	6.2
$\bar{x}$=	11.0	26.0	32.8	64.0	65.1	67.3	74.9	75.7	76.2

$s_{\bar{x}}$ = standard error (of the mean); s = standard deviation; $\bar{x}$ = mean percent germination
C1–3 = control means; L1–3 = leachate treatment means; E1–3 = extract treatment means

In all samples except one of *D. punctilobula* from New Jersey, the pH of the extracts was lower than that of the leachates (*Table 5*). The pH of the extracts and leachates from New Jersey populations did not differ greatly from that obtained from the Vermont material (*Table 6*). Although in all samples the pH of the leachates from Vermont populations was higher than in leachates from New Jersey, the difference probably is not enough to be significant. It can be deduced from *Table 6* that allelopathy is not strictly a low pH phenomenon. For example,

the leaf leachates and extracts from *O. cinnamomea* (New Jersey) exhibited very little phytotoxicity, yet they possessed the lowest pH.

As already mentioned, litter infusions of *O. cinnamomea* were found to be autotoxic (*Table 2*) only in the first count, indicating delayed germination. Such a delay could be important under field conditions, however, increasing the chances of attack by microbial pathogens which could cause damping off of the young prothalli, as is often the case with seeds.

Within the species studied in New Jersey, the pH values obtained for leaf-litter infusions were higher than expected when compared with those obtained for leaf leachates and extracts (*Table 6*).

TABLE 5. ALLELOPATHIC EFFECTS OF *D. PUNCTILOBULA* LEAF EXTRACTS AND LEACHATES EXPRESSED AS A PERCENTAGE OF SPORE GERMINATION AND EARLY GAMETOPHYTE GROWTH IN VERMONT AND NEW JERSEY POPULATIONS OF *O. CINNAMOMEA*. STATISTICAL ANALYSIS USING DUNCAN'S MULTIPLE RANGE TEST WITH A 0.05 SIGNIFICANCE LEVEL. (ALL EXTRACT (NOT LEACHATE) MEANS SIGNIFICANTLY DIFFERENT FROM ALL CONTROL MEANS IN VERMONT; ALL MEANS SIGNIFICANTLY DIFFERENT FROM ALL CONTROL MEANS IN NEW JERSEY.)

Vermont

Last count ($s_{\bar{x}}$ = 5.488)

Means	E2	E3	E1	L1	L2	L3	C1	C3	C2
s =	2.7	3.5	9.5	15.8	14.7	7.2	8.1	10.2	7.0
$\bar{x}$ =	27.8	33.2	38.8	47.2	53.6	67.6	76.8	80.5	81.3

New Jersey

First count ($s_{\bar{x}}$ = 4.569)

Means	E1	E3	E2	L2	L3	L1	C1	C2	C3
s =	10.4	8.1	10.6	7.4	4.2	8.8	7.9	7.5	3.0
$\bar{x}$ =	29.9	36.7	37.1	51.7	53.4	53.7	78.8	79.3	85.9

Last count ($s_{\bar{x}}$ = 4.608)

Means	E1	E2	E3	L3	L1	L2	C2	C3	C1
s =	12.2	12.3	5.3	3.9	6.1	4.4	8.3	6.1	8.2
$\bar{x}$ =	30.9	32.6	39.7	46.2	48.3	56.8	72.8	79.8	81.7

$s_{\bar{x}}$ = standard error (of the mean); s = standard deviation; $\bar{x}$ = mean per cent germination
C1–3 = control means; L1–3 = leachate treatment means; E1–3 = extract treatment means.

Frond litter was collected while the fronds were still standing, following the first killing frost in 1976. It is unknown whether or not it rained between the time of the first frost and the time of collection. If it did, this may account for the apparent lack of phytotoxicity in the infusions, since any water soluble phytotoxins could have been removed by the rainfall. The importance of the first rainfall after frond senescence already has been cited by Gliessman and Muller (1972), who studied the effects of *P. aquilinum* leaf litter extracts on *Avena fatua* and *Bromus rigida* radicle growth, and by Stewart (1975), who examined the effects of *P. aquilinum* litter extracts on seeds of *Rubus* sp. and Douglas-fir.

No inhibitory volatile compounds were detected in biologically significant levels from leaves removed from New Jersey or Vermont populations. *Dennstaedtia punctilobula* leaves are quite fragrant, particularly when crushed, but the substance producing this fragrance, presumably coumarin, had no apparent effect on spore germination.

Water-soluble inhibitors predominate in more humid or wet environments, according to the hypothesis developed by Whittaker (1970) relating toxin production to climate. Since the Vermont populations exist in a wetter environment than do the New Jersey ones (Climatological Data, U. S. Dept. of Commerce, NOAA, 1976–1977), this may partially explain the increased number of allelopathic and autotoxic interactions caused by water-soluble leachates and extracts. Volatile inhibitors, according to Whittaker (1970) and Muller (1970), would be most common in a hot, arid environment. The fact that none were found in either Vermont or New Jersey populations would also lend support to their theory relating toxin production to climate, as both areas are relatively moist. Summer temperatures in the study areas in Vermont and New Jersey were nearly identical (Climatological Data, NOAA, 1976–1977).

TABLE 6. pH VALUES OBTAINED FROM AQUEOUS LEAF EXTRACTS AND LEACHATES FOR NEW JERSEY AND VERMONT POPULATIONS, AND LITTER INFUSIONS FOR NEW JERSEY POPULATIONS.

	pH	
Species	NJ	VT
Osmunda cinnamomea		
leaf extract	5.40	5.20
leaf leachate	6.00	6.60
litter infusion	6.50	—
soil (5/77)	5.20	—
Osmunda claytoniana		
leaf extract	5.60	5.80
leaf leachate	6.20	6.65
litter infusion	6.75	—
soil (5/77)	6.40	—
Dennstaedtia punctilobula		
leaf extract	5.70	5.80
leaf leachate	5.50	6.00
litter infusion	5.95	—
soil (5/77)	5.90	—

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